# Spike Architecture Traits Associated With Type II Resistance to Fusarium Head Blight in Bread Wheat

**Maria F Franco**
Unidad Integrada Balcarce and CONICET
**Gladys Lori**
Universidad Nacional de la Plata Facultad de Ciencias Agrarias y Forestales
**Maria G Cendoya**
Unidad Integrada Balcarce
**Maria P Alonso**
Unidad Integrada Balcarce and CONICET
**Juan S Panelo**
Unidad Integrada Balcarce
**Ismael Malbrán**
Universidad Nacional de la Plata
**Nadia E Mirabella**
Unidad Integrada Balcarce
**Ana Clara Pontaroli** ( pontaroli.ana@inta.gob.ar )
Unidad Integrada Balcarce and CONICET https://orcid.org/0000-0002-2605-7668

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# SPIKE ARCHITECTURE TRAITS ASOCIATED WITH TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT IN BREAD WHEAT

Franco MF[ab1]; Lori, GA[cd]; Cendoya, G[a]; Alonso, MP[ab]; Panelo, JS[ac2]; Malbrán, I[bd]; Mirabella, NE[a]; Pontaroli, AC[ab3]

[a] Unidad Integrada Balcarce (Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata – Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria), CC 276 (7620), Balcarce, Argentina

[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (1425) CABA, Argentina

[c] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), CC 276 (7620) Balcarce, Argentina

[d] Centro de Investigaciones de Fitopatología (CIDEFI), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CC 31 (1900) La Plata, Buenos Aires, Argentina

ORCID: [1] 0000-0001-6039-7984; [2] 0000-0002-0075-0157

[3]Corresponding author. Ana Clara Pontaroli, CC 276 (7620) Balcarce, Argentina. Phone +54 2266 439103 ext 567/333; FAX +54 2266 439101; email pontaroli.ana@inta.gob.ar

## ABSTRACT

Fusarium head blight (FHB) remains a devastating disease in bread wheat (*Triticum aestivum* L.) and other small grains. Genetic resistance to FHB is a complex trait; in addition to active physiological resistance, plant developmental and morphological traits may indirectly affect disease progression and provide a passive mechanism of resistance. In this study, we investigated the relationship between FHB type II resistance and spike architecture traits in a recombinant inbred line (RIL) population of bread wheat. Disease resistance traits were FHB severity at 21 days post inoculation (dpi) and area under the disease progress curve (AUDPC). Spike architecture traits measured were rachis length, spike density, number of spikelets per spike, florets per spike and florets per spikelet.

The RIL population showed significant variation for all traits. Heritability values were moderate to high for FHB severity (0.69) and AUDPC (0.63) and high for the spike architecture traits (0.74 - 0.92). FHB severity and AUDPC showed a moderate and significant association with the number of florets per spike (r = 0.38 and r = 0.31, respectively) and with the number of florets per spikelet (r = 0.28 and r = 0.27, respectively), reflecting a greater spread of the fungus in spikes with higher floret number. These results suggest that the number of florets per spike and the number of florets per spikelet should be considered in FHB resistance breeding efforts, because selection of lines with higher number of florets could lead to a correlated selection response towards increased FHB levels under field conditions.

**Key words:** *Fusarium graminearum*, *Triticum aestivum* L., inflorescence traits, passive resistance.

## INTRODUCTION

Fusarium head blight (FHB), also known as head scab, is one of the most devastating diseases of wheat, frequently causing epidemics in many wheat-growing areas of the world (Lori et al. 2003; Mazzilli et al. 2007). This disease is prevalent in regions with prolonged warm and humid climatic conditions in the period from flowering to the soft dough stage of kernel development (Bai and Shaner 1994; Sutton 1982).

Although many *Fusarium* species can cause FHB, *Fusarium graminearum* Schwabe is one of the main pathogens associated with the disease in many countries of the world, including Argentina (Malbrán and Lori 2014; Schroeder and Christensen 1963; Sutton 1982). The fungus invades the spikes predominantly by direct penetration and colonizes the rachis and the spikelets. In this way, FHB leads to severe losses not only in grain yield but also in quality, decreasing seed germination and flour baking properties (McMullen et al. 1997). Damaging effects are further aggravated by the accumulation of mycotoxins produced by the fungus in the grains, which render them inappropriate for human or animal consumption (Kendrick 1992). Different control strategies such as crop rotation, tillage practices and fungicide application have been proposed to reduce the impact of FHB. However, these agronomic practices have a limited success. Therefore, the employment of FHB-resistant

53 cultivars is still the most reliable and consistent strategy for minimizing losses caused by the disease
54 (CIMMYT 2019).

55 Resistance to FHB is a complex trait: it is quantitatively inherited, significantly affected by
56 environmental conditions, and subjected to strong genotype-by-environment interactions (Bai and
57 Shaner 1994). Resistance to this disease is the result of passive and active mechanisms (Mesterhazy
58 1995; Rudd et al. 2001). Passive resistance includes morphological and developmental traits (for
59 example: plant height, spike architecture and flowering date) which alter conditions for initial
60 infection and subsequent fungal growth in the spike (Buerstmayr and Buerstmayr 2015). On the other
61 hand, active resistance mechanisms comprise biochemical pathways that produce compounds that
62 affect the pathogen during and/or after infection (Wiese 1987). The two main types of resistance to
63 FHB are resistance to initial infection (Type I) and resistance to spread of the pathogen within the
64 spike after infection (Type II) (Schroeder and Christensen 1963). In this way, considering that plant
65 architecture can play a significant role in disease resistance, establishing the relationship between
66 FHB resistance and architectural traits affecting disease development could be an advantageous
67 strategy for accelerating the development of resistant varieties (Zhu et al. 1999).

68 So far, several plant morphological and developmental traits have been investigated for their
69 association with FHB resistance. However, most of these studies have been carried out for FHB type I
70 resistance. Thus, resistance to initial infection has been correlated with flower opening and duration of
71 flower opening (Pugh et al. 1933; Zhang et al. 2018), extent of anther extrusion/retention (Buerstmayr
72 and Buerstmayr 2015; Kubo et al. 2013; Skinnes et al. 2005), plant height (Gervais et al. 2003; Steiner
73 et al. 2004) and flowering date (Buerstmayr et al. 2012; Steiner et al. 2004), among others. However,
74 little is known about the effect of morphological traits on type II resistance.

75 Since Fusarium head blight is a floral infection disease (Arthur 1891), once penetration occurs on
76 the inner surfaces of the lemma and palea or on the upper portion of the ovary, fungal hyphae spread
77 downwards to the rachilla and rachis node by inter- and intracellular growth. When the hyphae reach
78 the rachis, they spread upwards and downwards the entrance point through vascular bundles in the
79 rachis (Kang and Buchenauer 2000). Then, it may be hypothesized, for example, that wheat plants
80 which exhibit a longer rachis or lower inflorescence compactness have a lower disease progress and

severity by reducing the speed with which *Fusarium* hyphae can extend into the spike. In this way, the aim of this research was to study the relationships between FHB type II resistance and spike architecture traits, in order to enhance the current knowledge on this complex pathosystem from a breeding standpoint.

## MATERIALS AND METHODS

### Plant material

A biparental population of 126 recombinant inbred lines (RILs) was developed from a cross between 'Baguette 10' and 'Klein Chajá', two spring bread wheat cultivars of very different genetic background, agronomically adapted for cultivation in Argentina. This population was generated at the Instituto Nacional de Tecnología Agropecuaria (INTA) (Alonso et al. 2018; Martino et al. 2015; Mirabella et al. 2016). Both parental cultivars display medium FHB resistance level, but differ in spike architecture, as 'Baguette 10' has compact spikes whereas 'Klein Chajá' has lax spikes at maturity.

### Fusarium head blight resistance evaluation

#### Field experiments

The RIL population, the parental cultivars and four commercial checks were tested in field experiments at the INTA Balcarce Experimental Station (37°46'15'' S; 58°18' 24'' W; 112 m.a.s.l.), Buenos Aires province, Argentina during two consecutive years (2016 and 2017). In each crop season, two experiments were carried out, which differed in their sowing date by ca. one month; resulting in a total of four experiments (environments). Experiments were arranged as randomized complete block designs with two blocks (or replications). Sowing dates, sowing density and crop management were as described in Franco et al. (2020).

#### Inoculation technique and disease assessment

A macroconidial suspension of *F. graminearum* -isolate 'SP1'- was used for inoculation. This isolate was previously characterized by its aggressiveness and it was used in this study because it

caused the highest disease severity in two consecutive field tests (Malbrán et al. 2012; Malbrán et al. 2014). The macroconidial suspension was prepared as described by Malbrán et al. (2012) and the concentration was adjusted to ~100,000 spores $ml^{-1}$ using a haemocytometer.

Anthesis date, defined as the date in which 50% of the spikes of each individual plot was flowering -Zadoks growth stage 65 (Zadoks et al. 1974)-, was recorded for each genotype in all plots. Then, ten flowering spikes per plot were randomly picked and tagged with a numbered label for identification. The spikes were inoculated using the point inoculation (PI) technique as described in Franco et al. (2020).

Development of FHB symptoms was followed individually on each inoculated spike. The number of infected spikelets per spike was determined visually 12, 17, and 21 days post inoculation (dpi). FHB severity was estimated as the proportion of infected spikelets in a spike at 21 dpi (number of infected spikelets divided by the total number of spikelets per spike). The Area Under the Disease Progress Curve (AUDPC) was calculated for each spike, according to Shaner and Finney (1977) as:

$$AUDPC = \sum_{i=1}^{n} \frac{(S_{i+} S_{i+1})}{2} * (t_{i+1} - t_i)$$

where Si = disease severity at the $i^{th}$ observation, $t_i$ = days at the $i^{th}$ observation, and n = total number of observations.

**Evaluation of spike architecture traits**

Spike architecture traits were evaluated in all RILs and the parental cultivars. Rachis length, number of spikelets per spike and spike density were determined in all inoculated spikes. Rachis length was measured as the distance in cm between the top and the bottom node of the rachis. Number of spikelets per spike was the total number of fertile spikelets per spike. The average number of spikelets per cm of rachis length was calculated and used as an estimation of spike density. Number of florets per spike was counted on 15 randomly chosen spikes per plot from the two field experiments carried out in 2016 and one field experiment in 2017. The average number of florets per spikelet was estimated as the number of florets in the spike divided by the total number of spikelets in the spike.

## Statistical analysis

Statistical analyses were performed using R software (R Core Team 2013). All the data was analyzed fitting linear mixed models with the *lme* function from package *nlme* (Pinheiro et al. 2013). Residuals were tested for normality and homoscedasticity and a log-transformation was performed for FHB severity and AUDPC to normalize residuals. Models for logarithm of FHB severity and AUDPC were fitted considering the anthesis date as a fixed factor and genotypes and genotypes x environment interaction as random factors according to Franco et al. (2020).

Models for spike traits were fitted considering environment and block within environment as fixed effects and genotypes and genotype x environment interaction as random effects:

$$y_{ijk} = \mu + \alpha_j + \beta_{k(j)} + \tau_i + \gamma_{j(i)} + \varepsilon_{ijk}$$

Where $y_{ijk}$ is the logarithm of the response variable on block "*k*" of line "*i*" in the environment "*j*", $\mu$ is the mean value of the of response variable, $\alpha_j$ is the fixed effect of the environment "*j*" , $\beta_{k(j)}$ is the fixed effect of the block "*k*" in the environment "*j*" , $\tau_i$ is the random effect of line "*i*", $\gamma_{j(i)}$ is the random interaction effect between line "*i*" and environment "*j*", and $\varepsilon_{ijk(s)}$ is the random error of the observation on repetition "*k*" of line "*i*" in the environment "*j*" .

Assumptions on this model are: $\tau_i \sim N(0;\ \sigma_g^2)$, $\gamma_{j(i)} \sim N(0;\ \sigma_{ge}^2)$ and $\varepsilon_{ijk} \sim N(0;\ \sigma_{res}^2)$ all are independent of each other.

Sequential restricted maximum likelihood ratio tests were performed to determine the significances of the random effects of lines and lines by environment interactions. For all the variables, Best Linear Unbiased Predictors (BLUPs) were obtained for all RILs and parental cultivars. Variance components were estimated by the restricted maximum likelihood (REML) method (Milliken and Johnson 2001) and broad-sense heritabilities ($H^2$) were estimated from variance components according to Hallauer et al. (2010), as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{ge}^2}{e}\right) + \left(\frac{\sigma_{res}^2}{re}\right)}$$

where $\sigma_g^2$ is the genotypic variance, $\sigma_{ge}^2$ is the genotype x environment interaction variance, $\sigma_{res}^2$ is the error variance; e is the number of environments, and r is the number of replications per experiment.

To determine the significance of the genetic correlation between all the evaluated traits, Pearson correlation tests were performed with the obtained BLUPs.

## RESULTS

### FHB severity and AUDPC

Despite the great environmental variation observed between environments as well as between inoculation dates (**Fig. S1** and **Fig. S2**), FHB symptoms were present in all field experiments and evaluated genotypes, with an overall 70% incidence in conidia-inoculated spikes. Mean values of the parental cultivars and means, minimum and maximum scores and standard deviations of the RIL population for each experiment as well as for the overall mean across all experiments for FHB severity and AUDPC are presented in Table 1. Sequential restricted maximum likelihood ratio tests revealed highly significant variation due to genotypes for both variables ($p<0.01$) (Table 2). The RIL population showed continuous variation for these variables across the conducted experiments (**Fig. 1**). Averaged across experiments, FHB severity varied within the RIL population between 0.14 and 0.69, and the AUDPC, between 91.5 and 513.2. The parental cultivars exhibited an intermediate performance, although Baguette 10 showed consistently lower disease levels than did Klein Chajá, except for Exp. 1 in 2016. For both variables, transgressive segregation (i.e., the occurrence of RILs with more extreme values than those of the parents) was observed in all experiments.

**Table 1** Means, minimum (Min) and maximum (Max) values and standard deviations (SD) for FHB severity and AUDPC in the Baguette 10 x Klein Chajá RIL population (N = 126) and parental cultivars, as evaluated in four field experiments carried out in Balcarce, Argentina.

| Trait | Year | Experiment | Means of parental cultivars | | Values for RIL population | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Baguette 10 | Klein Chajá | Mean | Min | Max | SD |
| FHB Severity | 2016 | 1 | 0.69 | 0.46 | 0.38 | 0.05 | 1 | 0.24 |
| | 2016 | 2 | 0.40 | 0.46 | 0.51 | 0.06 | 1 | 0.2 |
| | 2017 | 1 | 0.18 | 0.44 | 0.24 | 0.05 | 0.85 | 0.15 |
| | 2017 | 2 | 0.25 | 0.36 | 0.27 | 0.05 | 1 | 0.18 |
| | **Overall mean** | | **0.38** | **0.43** | **0.34** | **0.05** | **1** | **0.23** |
| AUDPC | 2016 | 1 | 544.1 | 218.9 | 274.7 | 10.3 | 1088.0 | 202.5 |
| | 2016 | 2 | 350.9 | 262.7 | 368.4 | 11.0 | 924.4 | 193.8 |
| | 2017 | 1 | 135.0 | 280.8 | 167.3 | 11.9 | 892.7 | 112.6 |
| | 2017 | 2 | 192.6 | 259.9 | 214.4 | 8.5 | 855.1 | 128.9 |
| | **Overall mean** | | **305.7** | **255.6** | **252.2** | **8.5** | **1088.0** | **177.0** |

**Table 2** Sequential restricted maximum likelihood ratio tests to determine the significances of the random effects of lines and line by environment interactions for severity and AUDPC.

| Trait | Model[a] | Df[b] | AIC[c] | BIC[d] | logLik[e] | Test | L. Ratio[f] | p-value |
|---|---|---|---|---|---|---|---|---|
| FHB Severity | 1 | 29 | 1.704.002 | 1.843.400 | -8.230.008 | | | |
| | 2 | 28 | 1.709.290 | 1.843.881 | -8.266.449 | 1 vs. 2 | 728.811 | 0.0069 |
| | 3 | 27 | 1.816.911 | 1.946.696 | -8.814.558 | 2 vs. 3 | 10.962.179 | <.0001 |
| AUDPC | 1 | 29 | 1.864.524 | 2.003.922 | -9.032.619 | | | |
| | 2 | 28 | 1.864.798 | 1.999.389 | -9.043.989 | 1 vs. 2 | 227.400 | 0.1316 |
| | 3 | 27 | 1.933.160 | 2.062.945 | -9.395.801 | 2 vs. 3 | 7.036.224 | <.0001 |

[a] Model 1 is the complete model. Models 2 and 3, sequentially omit the random effect of line by environment interaction and random effect of the lines.
[b] Degrees of freedom
[c] Akaike information criterion
[d] Bayesian information criterion
[e] Log-Likelihood
[f] Likelihood-ratio test

## Spike architecture traits

The mean spike architecture traits' values of the parental cultivars and means, minimum and maximum scores and standard deviations of the RIL population for each experiment as well as for the overall mean across all experiments are presented in Table 3. The RIL population showed significant variation for rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet

across all experiments (Table 4). A bell-shaped frequency distribution was observed for each of the spike architecture traits evaluated in the population (**Fig. 2**). The parental cultivars showed intermediate values for all the variables. As it was expected, 'Baguette 10' exhibited a higher spike density than 'Klein Chajá', due to a higher number of spikelets in the spike and a shorter rachis. Also, 'Baguette 10' showed a lower number of florets per spikelet and per spike than did 'Klein Chajá'. Transgressive segregation was observed in all experiments.

**Table 3** Means, minimum (Min) and maximum (Max) values and standard deviations (SD) for spike architecture traits in the Baguette 10 x Klein Chajá RIL population (N = 126) and parental cultivars, as evaluated in four field experiments carried out in Balcarce, Argentina.

| Trait | Year | Experiment | Means of parental cultivars | | Values for RIL population | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Baguette 10 | Klein Chajá | Mean | Min | Max | SD |
| Rachis length | 2016 | 1 | 8.2 | 9.7 | 8.8 | 6.3 | 12.1 | 0.9 |
| | 2016 | 2 | 8.5 | 10.4 | 9.3 | 6.5 | 12.4 | 1.0 |
| | 2017 | 1 | 7.7 | 10.0 | 8.8 | 6.3 | 11.7 | 0.9 |
| | 2017 | 2 | 9.0 | 9.4 | 8.5 | 6.4 | 11.3 | 0.9 |
| | **Overall mean** | | **8.3** | **9.9** | **8.9** | **6.3** | **12.4** | **1.0** |
| Spike density | 2016 | 1 | 2.2 | 1.6 | 2.0 | 1.5 | 2.7 | 0.2 |
| | 2016 | 2 | 2.3 | 1.7 | 1.9 | 1.5 | 2.6 | 0.2 |
| | 2017 | 1 | 2.2 | 1.6 | 1.9 | 1.4 | 2.8 | 0.2 |
| | 2017 | 2 | 2.1 | 1.5 | 1.9 | 1.4 | 2.5 | 0.2 |
| | **Overall mean** | | **2.2** | **1.6** | **1.9** | **1.4** | **2.8** | **0.2** |
| Spikelets per spike | 2016 | 1 | 18.0 | 15.3 | 17.3 | 13.2 | 20.8 | 1.4 |
| | 2016 | 2 | 19.0 | 17.8 | 17.7 | 14.4 | 21.0 | 1.3 |
| | 2017 | 1 | 17.3 | 15.7 | 16.9 | 13.0 | 23.4 | 1.4 |
| | 2017 | 2 | 18.3 | 14.2 | 16.4 | 12.4 | 20.3 | 1.4 |
| | **Overall mean** | | **18.2** | **15.8** | **17.1** | **12.4** | **23.4** | **1.5** |
| Florets per spike | 2016 | 1 | 42.2 | 38.9 | 42.4 | 13.3 | 64.3 | 7.8 |
| | 2016 | 2 | 31.5 | 47.6 | 44.2 | 12.2 | 68.1 | 9 |
| | 2017 | 1 | 33.3 | 40.8 | 39.3 | 26.7 | 56.7 | 5.2 |
| | **Overall mean** | | **36.5** | **42.4** | **42.0** | **12.2** | **68.1** | **7.8** |
| Florets per spikelet | 2016 | 1 | 2.34 | 2.5 | 2.4 | 0.8 | 3.6 | 0.4 |
| | 2016 | 2 | 1.65 | 2.7 | 2.5 | 0.7 | 3.9 | 0.5 |
| | 2017 | 1 | 1.91 | 2.6 | 2.3 | 1.5 | 3.4 | 0.4 |
| | **Overall mean** | | **1.97** | **2.6** | **2.4** | **0.7** | **3.9** | **0.4** |

**Table 4** Sequential restricted maximum likelihood ratio tests to determine the significances of the random effects of lines and lines by environment interactions for the spike architecture traits.

| Trait | Model[a] | Df[b] | AIC[c] | BIC[d] | logLik[e] | Test | L. Ratio[f] | p-value |
|---|---|---|---|---|---|---|---|---|
| Rachis | 1 | 11 | 2.330.097 | 2.383.961 | -1.154.049 | | | |
| length | 2 | 10 | 2.333.287 | 2.382.254 | -1.156.643 | 1 vs 2 | 518.951 | 0.0227 |
| | 3 | 9 | 2.634.656 | 2.678.726 | -1.308.328 | 2 vs 3 | 30.336.916 | <.0001 |
| Spike | 1 | 11 | -12.522.349 | -11.983.713 | 6.371.175 | | | |
| density | 2 | 10 | -12.499.499 | -12.009.830 | 6.349.750 | 1 vs 2 | 42.850 | 0.0385 |
| | 3 | 9 | -6.146.743 | -5.706.041 | 3.163.372 | 2 vs 3 | 6.372.756 | <.0001 |
| Spikelets | 1 | 11 | 3.147.756 | 3.201.620 | -1.562.878 | | | |
| per spike | 2 | 10 | 3.155.215 | 3.204.182 | -1.567.608 | 1 vs 2 | 94.590 | 0.0021 |
| | 3 | 9 | 3.486.162 | 3.530.232 | -1.734.081 | 2 vs 3 | 3.329.469 | <.0001 |
| Florets | 1 | 9 | 4.697.509 | 4.738.456 | -2.339.755 | | | |
| per spike | 2 | 8 | 4.701.134 | 4.737.531 | -2.342.567 | 1 vs 2 | 562.414 | 0.0177 |
| | 3 | 7 | 4.844.716 | 4.876.564 | -2.415.358 | 2 vs 3 | 14.558.264 | <.0001 |
| Florets | 1 | 9 | 6.671.720 | 7.079.892 | -3.245.860 | | | |
| per | 2 | 8 | 6.724.175 | 7.086.994 | -3.282.087 | 1 vs 2 | 724.547 | 0.0071 |
| spikelet | 3 | 7 | 8.317.668 | 8.635.135 | -4.088.834 | 2 vs 3 | 16.134.930 | <.0001 |

[a] Model 1 is the complete model. Models 2 and 3, sequentially omit the random effect of line by environment interaction and random effect of the lines.
[b] Degrees of freedom
[c] Akaike information criterion
[d] Bayesian information criterion
[e] Log-Likelihood
[f] Likelihood-ratio test

## Variance components and heritabilities

Variance component analysis by REML revealed that $\sigma_g^2$ was greater than $\sigma_{ge}^2$ for all variables (Table 5). Medium to high heritability values were observed for FHB severity (0.69) and AUDPC (0.63), indicating than a high portion of the observed phenotypic variation was caused by the genotypic component. As expected, heritability values were high for spike architecture traits (between 0.74 and 0.92).

**Table 5** Variance component estimates (genotypic, genotype x environment interaction and residual variances) and broad-sense heritability ($H^2$) for the analyzed traits.

| Trait | Genotypic variance ($\sigma_g^2$) | Genotype x environment interaction variance ($\sigma_{ge}^2$) | Residual variance ($\sigma_{res}^2$) | Broad-sense heritability ($H^2$) |
|---|---|---|---|---|
| FHB severity [a] | 0.09 | 0.04 | 0.24 | 0.69 |
| AUDPC[a] | 0.08 | 0.03 | 0.32 | 0.63 |
| Rachis length[a] | 0.33 | 0.05 | 0.41 | 0.84 |
| Spike density[a] | 0.02 | 0.001 | 0.01 | 0.92 |
| Spikelets per spike[a] | 0.81 | 0.16 | 0.91 | 0.84 |
| Florets per spike[b] | 19.9 | 5.28 | 31.40 | 0.74 |
| Florets per spikelet[a] | 0.07 | 0.02 | 0.09 | 0.81 |

[a] Data of 126 RILs, 4 environments (2 years x 2 experiments), two blocks within experiment
[b] Data of 126 RILs, 3 environments (2 experiments in 2016 and 1 experiment in 2017)

## Genetic correlation analysis

Correlation coefficients between BLUPs of FHB severity, AUDPC, rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet are shown in Table 6. The strongest genetic correlation coefficient (0.94) was detected between FHB severity and AUDPC.

Both variables showed a moderate and significant association with the number of florets per spike (r = 0.38 and r = 0.31, respectively) and with the number of florets per spikelet (r = 0.28 and r = 0.27, respectively), reflecting a greater spread of the fungus in spikes with higher floret number. Also, a significant, positive correlation (r = 0.59) was found between the number of florets per spike and number of florets per spikelet. Rachis length, number of spikelets per spike and spike density had no influence on FHB severity or AUDPC. As it was expected, the number of spikelets per spike was positively correlated with both rachis length (r = 0.58) and spike density (r = 0.27).

**Table 6** Genetic correlation coefficients between FHB severity, AUDPC, rachis length, spike density, spikelets per spike, florets per spike and florets per spikelet in the Baguette 10 x Klein Chajá RIL population, evaluated in four environments at Balcarce, Argentina (N = 126).

| | FHB severity | AUDPC | Rachis length | Spike density | Spikelets per spike | Florets per spike |
|---|---|---|---|---|---|---|
| AUDPC | 0.94*** | | | | | |
| Rachis length | 0.12 | 0.10 | | | | |
| Spike density | -0.13 | -0.13 | -0.61*** | | | |
| Spikelets per spike | 0.01 | 0.03 | 0.58*** | 0.27** | | |
| Florets per spike | 0.38*** | 0.31** | 0.29*** | -0.08 | 0.27* | |
| Florets per spikelet | 0.28** | 0.27* | -0.01 | -0.19* | -0.19* | 0.59*** |

* Correlation significant at the 0.05 level;
** Correlation significant at the 0.01 level;
*** Correlation significant at the 0.001 level

## DISCUSSION

Passive resistance mechanisms act through expression of morphological and developmental features which alter conditions for initial infection and allow the plant to avoid contact with the pathogen or prevent the disease development once the contact has taken place (Mesterhazy 1995). To date, most studies dealing with the association between plant morphological/developmental traits and FHB resistance have focused on type I resistance (Gervais et al. 2003; Pugh et al. 1933; Steiner et al. 2004; Zhang et al. 2018). However, little has been investigated about the effect of passive mechanisms on type II resistance.

In a recent work, we found that the anthesis date is correlated with type II resistance and that the prevailing environmental conditions during this stage affect the F. *graminearum* spread within the spike (Franco et al. 2020). Thus, considering this trait allows a more precise and objective characterization of the level of FHB type II resistance. In the same way, gaining insight into the associations between type II resistance to FHB and the architecture of the spike may lead to a

267 reduction of the "background noise" of traits that potentially influence the disease development, hence
268 increasing FHB resistance through the introgression of such desirable traits.

269 In this study, FHB type II resistance was evaluated in a RIL population of bread wheat, developed
270 from the cross between two cultivars with moderate level of resistance to FHB and contrasting spike
271 architecture, after implementing a precise point inoculation technique at anthesis under field
272 conditions. Also, several spike architectural traits which might alter fungal colonization of the spike
273 were evaluated. The RIL population used in this study showed large genetic variation for both FHB
274 severity and AUDPC across all experiments. A continuous distribution with transgressive segregation
275 towards lower and higher values for the two variables was observed. The population also segregated
276 for the spike architecture traits and showed a continuous normal frequency distribution with
277 transgressive variation for all the evaluated attributes. This supports the quantitative inheritance nature
278 of all the studied attributes. The high broad-sense heritability values obtained for FHB severity ($H^2$ =
279 0.69), AUDPC ($H^2$ = 0.63) and all the spike traits ($H^2$ between 0.74 and 0.92) indicate that a large
280 proportion of the variation among the evaluated lines was due to genetic effects, particularly
281 considering that the experiments performed in this study spanned a wide array of environmental
282 conditions.

283 In relation to the associations evaluated here, the strong and significant level of genetic correlation
284 between the two variables associated with the disease, FHB severity and AUDPC (r = 0.94), coincides
285 with that documented by other authors (Malbrán et al. 2012; Mourelos et al. 2014) and strengthen the
286 idea that both traits are under the same genetic control (Groth et al. 1999).

287 In this study, FHB severity and AUDPC were moderately, positively, and significantly associated
288 with both the total number of florets per spike and the number of florets per spikelet. Likewise, the
289 correlation observed between the number of florets per spike and per spikelet was high and
290 significant. To the best of our knowledge, this is the first report in which these genetic correlations are
291 evidenced in bread wheat. The associations found here could be explained by the fact that these spike
292 traits can provide a microclimate of high humidity in the spike, favoring the fungal spread and
293 sporulation within the spike, increasing the level of disease.

294 Spike density is a function of two traits -rachis length and number of spikelets per spike- (Faris et
295 al. 2014). In the present study, spike density was not correlated with severity or with AUDPC. To
296 date, the effect of spike density on FHB resistance is unclear. On the one hand, Buerstmayr et al.
297 (2011) and Steiner et al. (2004) have found that laxer spikes were significantly associated with an
298 increase in FHB type II resistance, arguing that genotypes with more compact spikes have a faster
299 disease dissemination than do genotypes with lax spikes due to the microclimate conditions that are
300 generated in the more compact spikes (Rudd et al. 2001). On the other hand, there are some reports
301 indicating variable associations between these attributes depending on the population studied
302 (Buerstmayr et al. 2012).
303 No association between rachis length and FHB type II resistance was detected in the present study.
304 This is consistent with results reported by Somers et al. (2003) who, studying different associations
305 between FHB and morphological and phenological variables under controlled conditions, found no
306 correlation between spike length and FHB type II resistance. However, Buerstmayr et al. (2011)
307 reported a negative and significant association ($r = -0.27$) between spike length and AUDPC.
308 The number of spikelets on the spike was not correlated either with FHB severity or AUDPC.
309 These results are in agreement with that reported by Buerstmayr et al. (2011), who, studying the
310 association between different morphological characters and type II resistance to FHB in a population
311 of *Triticum macha* Dek.et Men. x *T. aestivum* L., also found no significant association between the
312 number of spikelets and the progression of the disease. Similarly, Liu et al. (2007) reported lack of
313 correlation between the number of spikelets per spike and type II resistance to FHB.
314 It is important to highlight that while correlation coefficients between variables reported in the
315 bibliography are generally estimated from the means of the variables studied (phenotypic
316 correlations), in this study the correlations were calculated using the BLUPs for each variable (i.e.,
317 genetic correlations). An important property of BLUPs is the shrinkage towards the mean, which is
318 often a desirable statistical property as it increases precision, while maximizing the correlation of true
319 genotype values and predicted genotype values (Piepho et al. 2008).
320 In summary, the results shown here suggest that the number of florets per spike and the number of
321 florets per spikelet should be considered in FHB resistance breeding efforts, because selection of lines

with higher number of florets could lead to a correlated selection response towards increased FHB levels under field conditions.

## DECLARATIONS


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### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Author contributions

MFF, ACP, GAL and IM designed the study. Field experiments were designed by MGC, ACP and MFF. Inoculum was prepared by IM and field experiments were performed by MFF, JSP, MPA, ACP and NEM. Statistical analyses were performed by MFF with the contribution of MGC; production of figures and tables was performed by MFF with the contribution of ACP and MGC. The manuscript was written by MFF with the contribution of ACP, MGC and GL. All authors read and approved the final manuscript.

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454 **Figure legends**
455
456 **Fig. 1** Frequency distribution of (a) FHB severity and (b) Area Under the Disease Progress Curve
457 (AUDPC) -average of four field experiments carried out in Balcarce, Argentina- in the Baguette 10 x
458 Klein Chajá RIL population (N = 126). The values of the parental cultivars are indicated with arrows

459 **Fig. 2** Frequency distribution of (a) Rachis length, (b) Spike density, (c) Spikelets per spike, (d)
460 Florets per spike and (e) Florets per spikelet -average of four field experiments carried out in
461 Balcarce, Argentina- in the Baguette 10 x Klein Chajá RIL population (N = 126). The values of the
462 parental cultivars are indicated with arrows
463

464 **Supplementary figures**

465

466 **Fig. S1** Maximum, minimum and medium temperature during the anthesis period in Experiment 1 in
467 2016 (a), Experiment 2 in 2016 (b), Experiment 1 in 2017 (c) and Experiment 2 in 2017 (d)

468 **Fig. S2** Rainfall (bars) and relative humidity (lines) during the anthesis period in Experiment 1 in
469 2016 (a), Experiment 2 in 2016 (b), Experiment 1 in 2017 (c) and Experiment 2 in 2017 (d)
470

471

# Figures

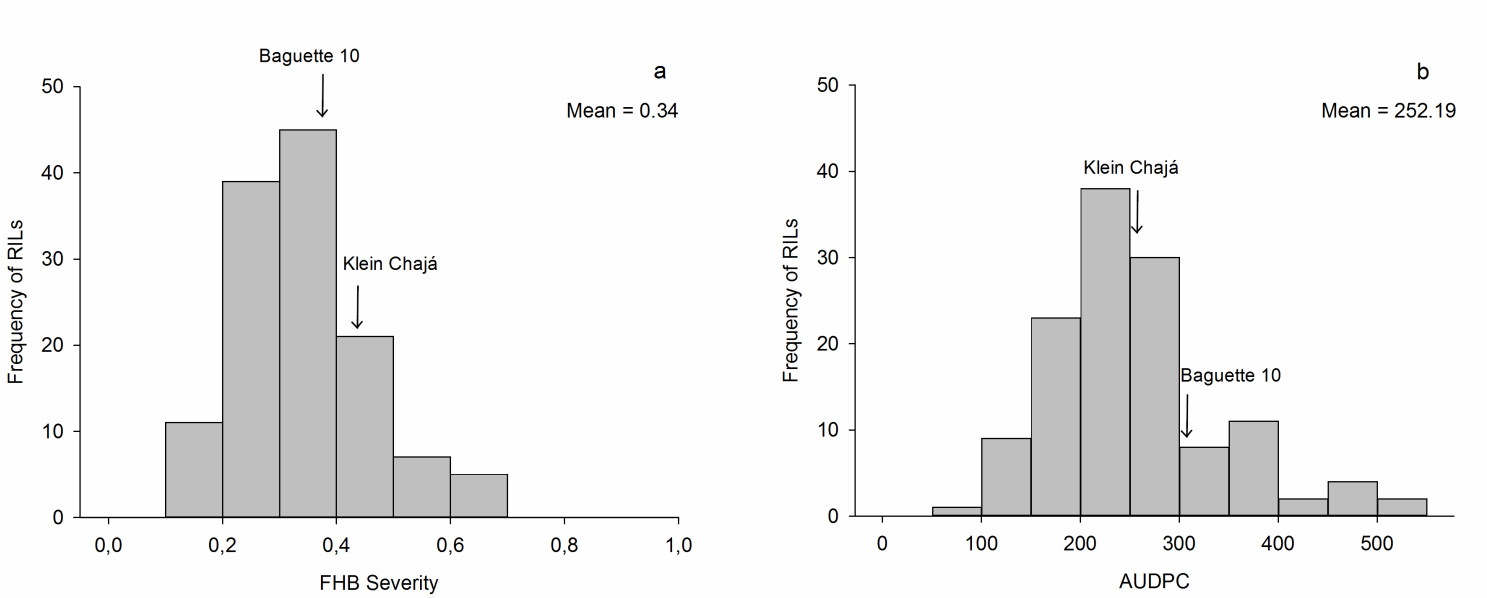
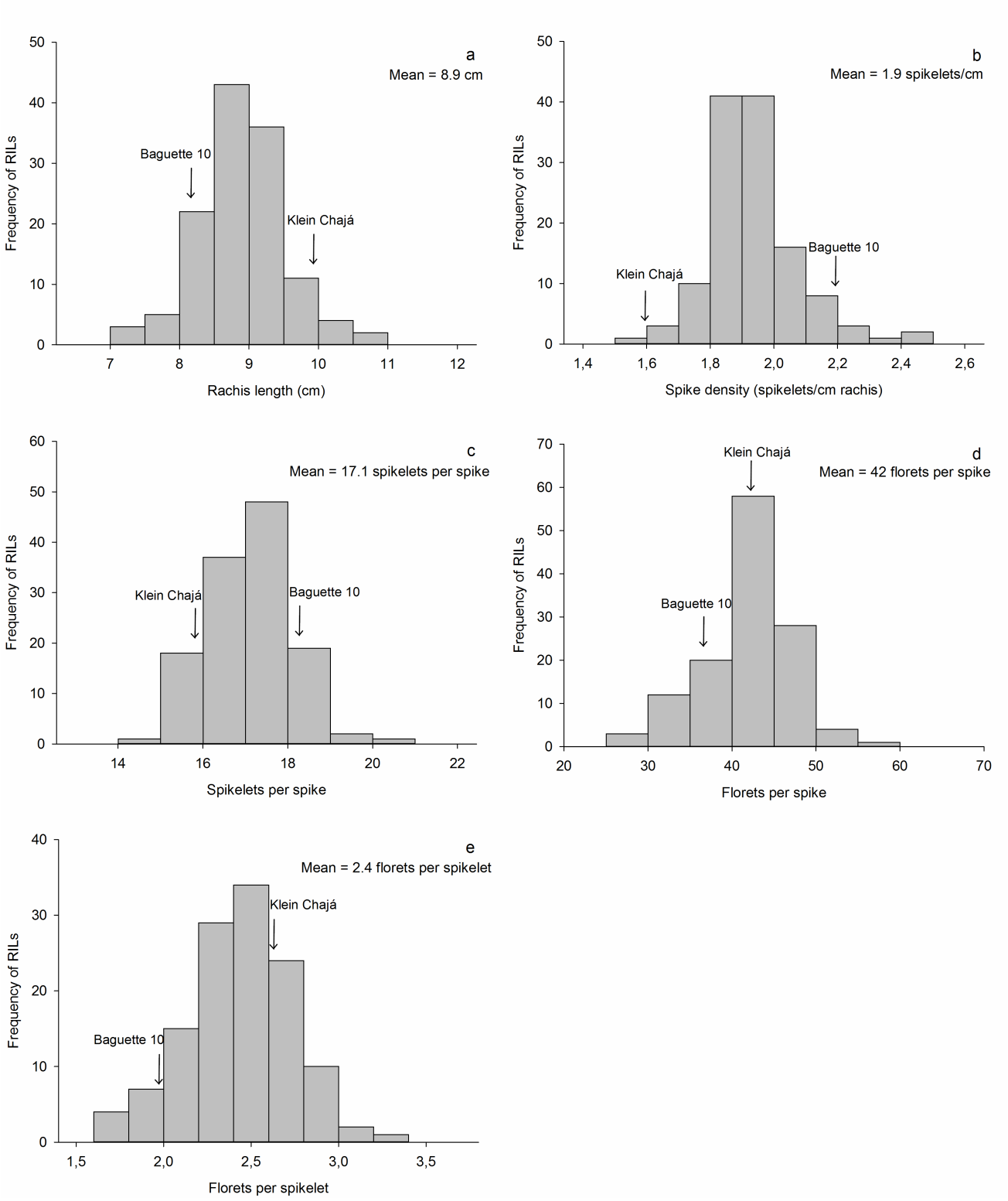



**Figure 1**

Frequency distribution of (a) FHB severity and (b) Area Under the Disease Progress Curve (AUDPC) - average of four field experiments carried out in Balcarce, Argentina- in the Baguette 10 x Klein Chajá RIL population (N = 126). The values of the parental cultivars are indicated with arrows




## Figure 2

Frequency distribution of (a) Rachis length, (b) Spike density, (c) Spikelets per spike, (d) Florets per spike and (e) Florets per spikelet -average of four field experiments carried out in Balcarce, Argentina- in the Baguette 10 x Klein Chajá RIL population (N = 126). The values of the parental cultivars are indicated with arrows

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